AMENDMENTS TO THE CLAIMS

1. (Original) A D-aminoacylase having the following enzymological properties:

(a) action: acting on a N-acetyl-D-amino acid to produce a D-amino acid;

(b) molecular weight: about 55,000 daltons when determined by SDS-polyacrylamide gel electrophoresis;

(c) isoelectric point: an isoelectric point of 5.3 when measured by two-dimensional electrophoresis for denatured system;

(d) substrate specificity: acting on N-acetyl-D-amino acids, and in particular on N-acetyl-D-valine, but not on N-acetyl-L-amino acids;

acting on substrates such as N-acetyl-D-valine, N-acetyl-D-leucine, N-acetyl-D-methionine, N-acetyl-D-tryptophan, N-acetyl-D-phenylalanine, and N-acetyl-D-tyrosine, but not on N-acetyl-L-valine, N-acetyl-L-leucine, N-acetyl-L-methionine, N-acetyl-L-tryptophan, N-acetyl-L-phenylalanine, and N-acetyl-L-tyrosine;

(e) thermostability: relatively stable at 4°C to 30°C when heated at pH 8.5 for 1 day;

(f) optimal temperature: optimally active at 37°C when reacted at pH 8 for 30 minutes;

(g) pH stability: stable near pH 9, and relatively stable near pH 7 to 10 when heated at a temperature of 30°C for 1 day;

(h) optimal pH: optimally active near pH 8 to 8.5 when reacted at 37°C;

(i) effects of metal ions: activity is inhibited by Mn^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+} each at 1 mmol/L; and

(j) effects of inhibitors: activity is inhibited by dithiothreitol, 2-mercaptoethanol, o-phenanthroline, and L-cysteine each at 5 mmol/L.

2. (Original) A D-aminoacylase comprising a protein defined in either one of (a) and (b):

(a) a protein comprising the amino acid sequence of SEQ ID NO.2; and

(b) a protein comprising an amino acid sequence wherein substitution, deletion, or addition of one to several amino acids has occurred in the amino acid sequence of SEQ ID NO.2, and having D-aminoacylase activity.

3. (Original) A gene coding a D-aminoacylase comprising a protein defined in either one of (a) and (b):

(a) a protein comprising the amino acid sequence of SEQ ID NO.2; and

(b) a protein comprising an amino acid sequence wherein substitution, deletion, or addition of one to several amino acids has occurred in the amino acid sequence of SEQ ID NO.2, said protein having D-aminoacylase activity.

4. (Original) The gene according to claim 3 comprising a DNA defined in either one of (c) and (d):

(c) a DNA comprising the base sequence of SEQ ID NO. 1; and

(d) a DNA which hybridizes under stringent conditions with the DNA comprising the base sequence which is complimentary to the base sequence of SEQ ID NO. 1, and which codes for a protein having D-aminoacylase activity.

5. (Original) A microorganism of genus Defluvibacter which produces a D-aminoacylase that produces a D-amino acid from a N-acetyl-D,L-amino acid or a N-acetyl-D-amino acid.

6. (Original) The microorganism according to claim 5 which has been designated Defluvibacter sp. A131-3 and deposited as FERM BP-08563.

7. (Currently Amended) The microorganism according to claim 5 ~~or 6~~ which produces ~~the~~ a D-aminoacylase ~~of claim 1 or 2~~ having the following enzymological properties:

(a) action: acting on a N-acetyl-D-amino acid to produce a D-amino acid;

(b) molecular weight: about 55,000 daltons when determined by SDS-polyacrylamide gel electrophoresis;

(c) isoelectric point: an isoelectric point of 5.3 when measured by two-dimensional electrophoresis for denatured system;

(d) substrate specificity: acting on N-acetyl-D-amino acids, and in particular on N-acetyl-D-valine, but not on N-acetyl-L-amino acids;

acting on substrates such as N-acetyl-D-valine, N-acetyl-D-leucine, N-acetyl-D-methionine, N-acetyl-D-tryptophan, N-acetyl-D-phenylalanine, and N-acetyl-D-tyrosine, but not on N-acetyl-L-valine, N-acetyl-L-leucine, N-acetyl-L-methionine, N-acetyl-L-tryptophan, N-acetyl-L-phenylalanine, and N-acetyl-L-tyrosine;

(e) thermostability: relatively stable at 4°C to 30°C when heated at pH 8.5 for 1 day;

(f) optimal temperature: optimally active at 37°C when reacted at pH 8 for 30 minutes;

(g) pH stability: stable near pH 9, and relatively stable near pH 7 to 10 when heated at a temperature of 30°C for 1 day;

(h) optimal pH: optimally active near pH 8 to 8.5 when reacted at 37°C;

(i) effects of metal ions: activity is inhibited by Mn^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+} each at 1 mmol/L; and

(j) effects of inhibitors: activity is inhibited by dithiothreitol, 2-mercaptoethanol, o-phenanthroline, and L-cysteine each at 5 mmol/L.

8. (Currently Amended) A method for producing the D-aminoacylase of claim 1 ~~or 2~~, comprising

~~wherein the~~ cultivating a microorganism of genus Defluvibacter which produces a D-aminoacylase that produces a D-amino acid from a N-acetyl-D,L-amino acid or a N-acetyl-D-amino acid ~~of any one of claims 5 to 7 is cultivated~~, and

recovering the D-aminoacylase ~~is recovered~~ from the culture.

9. (Currently Amended) A method for producing a D-amino acid ~~wherein~~ comprising

reacting the D-aminoacylase of claim 1 ~~or 2 is acted on~~ with a N-acetyl-D,L-amino acid or a N-acetyl-D-amino acid.

10. (New) The microorganism according to claim 7 which has been designated Defluvibacter sp. A131-3 and deposited as FERM BP-08563.

11. (New) The microorganism according to claim 5 which produces a D-aminoacylase comprising a protein defined in either one of (a) and (b):

(a) a protein comprising the amino acid sequence of SEQ ID NO.2; and

(b) a protein comprising an amino acid sequence wherein substitution, deletion, or addition of one to several amino acids has occurred in the amino acid sequence of SEQ ID NO.2, and having D-aminoacylase activity.

12. (New) The microorganism according to claim 11 which has been designated Defluvibacter sp. A131-3 and deposited as FERM BP-08563.

13. (New) A method for producing the D-aminoacylase of claim 2, comprising

cultivating a microorganism of genus Defluvibacter which produces a D-aminoacylase that produces a D-amino acid from a N-acetyl-D,L-amino acid or a N-acetyl-D-amino acid, and

recovering the D-aminoacylase from the culture.

14. (New) A method for producing a D-amino acid comprising

reacting the D-aminoacylase of claim 2 with a N-acetyl-D,L-amino acid or a N-acetyl-D-amino acid.